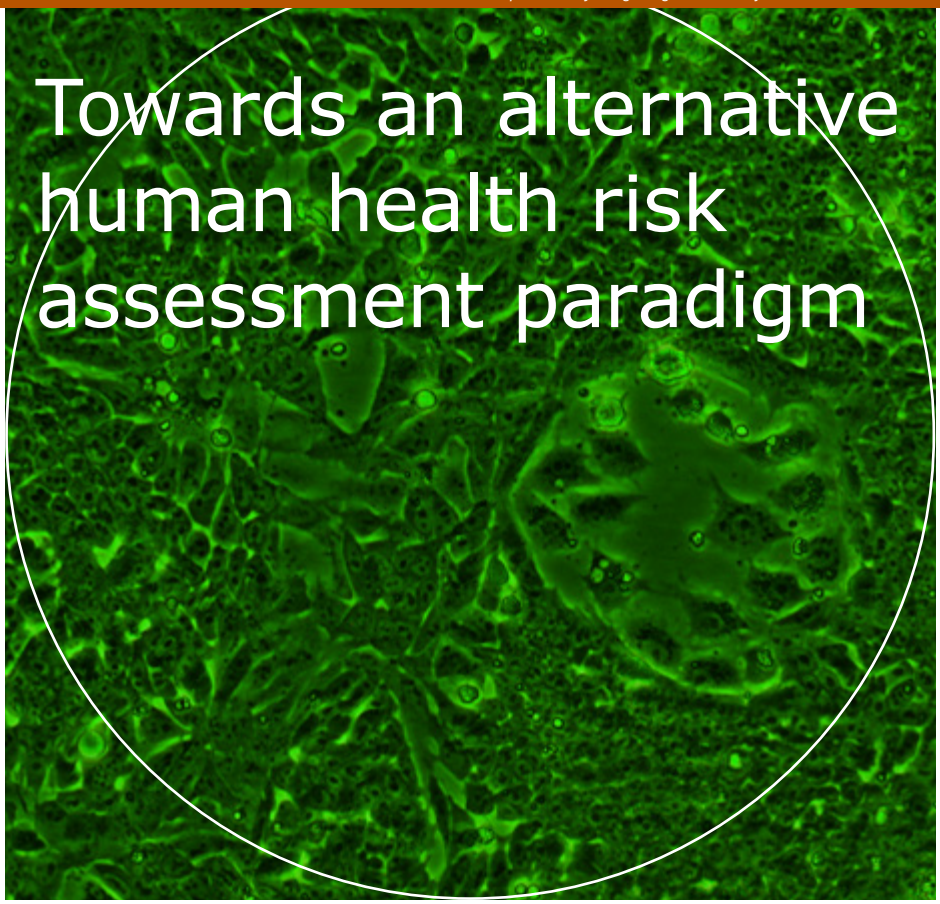

Towards an alternative human health risk assessment paradigm

Prof.dr Peter J. Boogaard

Inaugural lecture upon taking up the position of Special Professor of Environmental Health and Human Biomonitoring at Wageningen University & Research on 19 October 2017

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Towards an alternative human health risk assessment paradigm

Distinguished colleagues, dear family and friends,

Many people worry about chemicals in their environment. They worry in particular about being exposed to these chemicals through the food they eat, the air they breathe, the water they drink, and through all kind of items they use in their daily life. This societal concern is mainly about man-made chemicals which are often thought to be more toxic and to cause more adverse health effects than 'natural' chemicals.

We have seen a good example of these concerns last summer with the fipronil-contaminated eggs. Of course - according to the existing legislation - there should not have been any fipronil in the eggs. In the weeks after the fipronil was found to be present, more than 2.5 million chicken were culled and even more eggs were destroyed. Although the presence of fipronil in the eggs was not allowed, the question remained whether the levels that were found posed a threat to the health of people consuming them. Initially it was communicated that upon consumption of a few eggs there would be an acute health risk, while later on this was denied. The question is: how do we know? How do we know whether a certain level of a chemical in a food product is a health risk? If we do not yet know, how do we then assess the human health risk? This is the type of question toxicology should answer.

Coming back to the general concern over man-made chemicals, we have seen over the past number of years two specific developments. Firstly, there is an increasing concern about chemicals that may interact with hormonal systems and may cause adverse health effects, resulting especially in reproductive toxicity. Such reproductive toxicants may either have an adverse effect on fertility or may affect the normal development of the unborn child. Chemicals which are suspect of having hormone-like properties are usually referred to as 'endocrine disruptors' or 'endocrine

modulators' [1-4]. Secondly, there is an increasing concern that even if chemicals alone may not have any adverse health effect at a certain exposure level, they may exert unwanted effects when concomitant exposure occurs to other chemicals [5, 6]. This is referred to as 'combination toxicology', 'mixture toxicology' or, by some activist groups, as exposure to 'toxic cocktails'.

In this respect, it should be realized that there is a large category of chemicals that by nature are not simple substances, but rather complex substances that may comprise dozens to millions of different molecules. Exposure to these substances is therefore always a 'combination exposure'. On a regulatory level, in national and European chemicals' legislation, such complex chemicals are referred to as UVCB substances, which is an abbreviation that stands for substances of Unknown or Variable composition, Complex reaction products, or Biological materials. UVCB substances form about a third of the volume of chemicals put on the EU market annually and comprise substances such as fatty acid derivatives, fragrances, colourants, metal salts, petroleum hydrocarbons, and enzymes.

It is the task of toxicologists to determine whether exposure to substances, be it in isolation or in complex mixtures, poses a human health risk. However, due to their complex and variable composition toxicological testing of these UVCB substances is often difficult. So, how do we perform the human health risk assessment of such complex UVCB substances? To answer that question, we go back to what I would call 'the First Law of Toxicology', which was formulated by a Swiss physician born about a year after Columbus first reached America in 1492. He was born close to the village of Einsiedeln in Switzerland as Philippus Aureolus Theophrastus Bombast von Hohenheim. He became a very famous physician in his time for at least two reasons. The first being the fact that he published in German instead of in Latin – which did not really go well with his fellow physicians – and the second being that he was a very good observer and noted that many of the classical ideas about medicine had no basis and were factually wrong. In any case, he was considered a great physician, comparable to Aulus Cornelius Celsus, the famous Roman physician from the early first century. Hence Theophrast Bombast von Hohenheim got the nickname Paracelsus, 'comparable to Celsus', and that is the name by which he is now commonly known to us. As said, he had regular disputes with his fellow physicians about concepts handed down from classical times and he would then usually write a so-called 'Defension' to defend his case. In 'Die Dritte Defension', which after his death was bundled with his other defenses in 'Septem Defensiones', we can read the text (see Figure 1) which made him the 'father of toxicology': "*Was ist das nit gifft ist? alle ding sind gifft/und nichts ohn gift/Allein die dosis macht das ein ding kein gift ist*" – or, in English, "*What is that is no poison? All things are poison/and nothing without poison.*

Only the dose makes that a thing is not poison." In some text books it is suggested that this knowledge was lost and only rediscovered in our time, but that is not true as can be seen from the Latin annotation in the margin "*Nil sine veneno praesertim dosi non servari*" or "*Nothing is without poison if the dose is not taken into account*" that indicates that the importance of this observation was well recognized in the 16th century as well. In modern toxicological terms: risk is a function of hazard and dose, whilst the dose is a function of exposure and time.

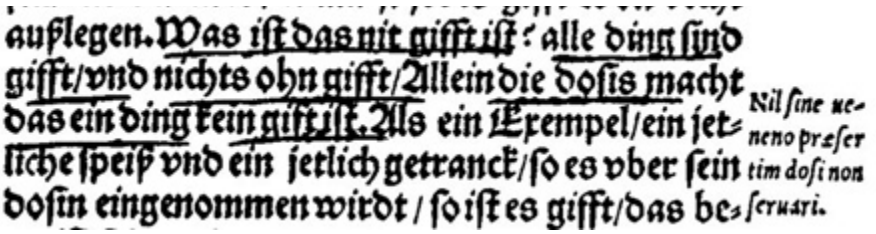
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Figure 1: The famous quote about toxicity from Paracelsus in 'Das Buch Paragranum'[7].

So, chemicals are not toxic as such, but the dose (or rather the exposure, that is the amount we are exposed to over a period of time) determines whether a chemical exerts a toxic effect or not. The dose makes the poison and this is very well illustrated by the text on the cartons of 'rum-rozijnen vla', a popular Dutch dessert of custard containing raisins soaked in real rum. According to the information on the package the custard contains about 0.2% of alcohol (ethanol). Ethanol is a dangerous chemical that is a known human category 1 carcinogen and also a known human category 1 reproductive toxicant, which implies that there is no scientific doubt on the fact that it can cause both cancer and birth defects in humans. However, we usually only worry about its effects on our capability to drive a vehicle safely. This is apparently also the concern of the manufacturer of this dessert since on the back of the packaging is the advice not to drive a vehicle after consumption of 138 litres of the custard. A clear illustration that the dose makes the poison…

This concept defines the current health risk assessment paradigm that implies that we conduct a hazard assessment, we determine the dose-response relationship and combine this information with information on the exposure to characterise the health risk. Depending on the outcome of this risk assessment we may need to manage the risk, usually by reducing the exposure (Figure 2).

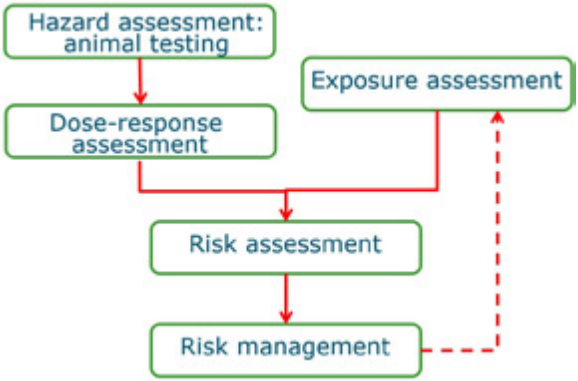

Figure 2. The generally accepted standard human health risk assessment scheme.

The hazard assessment is typically based on results obtained from animal testing. However, there are many issues with using animals for toxicity testing, both scientific and ethical issues. To address some of these issues we should move to 21st century toxicology as described in the report of the US National Academy of Sciences "Toxicology in the 21st century – a vision and strategy" [8]. This actually requires changing our current human health risk assessment paradigm, described above, in several ways. Firstly, and that will be the first general theme of the research I will undertake at the Division of Toxicology in Wageningen, we need to move away from mammalian-based *in vivo* testing in hazard assessment to *in vitro* hazard assessments using cell-based test systems, preferably human or humanized cell-based test systems. Secondly, and that will be the second theme of research, we will need to translate the *in vitro* data to the *in vivo* situation, to correlate the concentration in a test tube to the concentration in a human body and the corresponding dose level. To that purpose, we intend to apply human biomonitoring in combination with physiologically-based kinetic modelling, so-called PBK modelling. PBK modelling applies mathematical descriptions of mammalian physiology in combination with the metabolism and kinetics of substances. The combination of both leads to an alternative health risk assessment scheme, in which animal-based testing is replaced by animal-free testing and in which external exposure assessment is replaced by internal exposure assessment using human biomarkers (Figure 3).

Figure 3. The alternative human health risk assessment scheme with animal-free hazard assessment and internal exposure assessment.

Let us first address a research project we are currently working on that deals with the first theme. In this project, we develop and apply alternative, animal-free test systems to assess the prenatal developmental toxicity of UVCB substances. The various steps in the reproduction process can be described with the reproductive cycle. This cycle can be broken into three distinct parts (Figure 4).

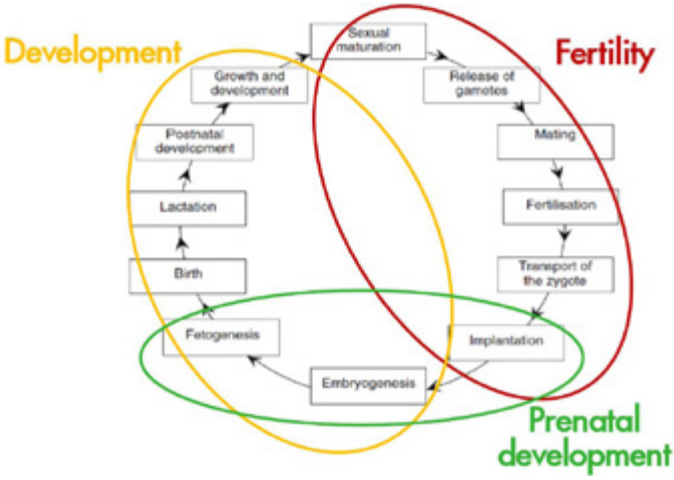

Figure 4. The reproductive cycle. Inside the red ellipse are all the stages that determine the fertility, in the yellow ellipse are the processes that describe the normal development from an embryo to an adult organism and in the green ellipse are the processes that occur in the womb from implantation of the embryo until birth (prenatal development).

The first describes fertility and goes from adult animals that produce gametes, mate, produce fertilized eggs that are implanted in the uterus. The second part describes development and starts with the embryogenesis of the implanted gametes and subsequent foetogenesis, birth, and then the whole period from early life during lactation to the further development to an adult animal. An important subsection is

the prenatal developmental phase that describes the period starting with the implanted gametes until birth and which is known to be highly susceptible to disturbances caused by external factors, including chemical exposure.

There is a large number of validated study designs to investigate the toxicity of substances on the various phases of reproduction. Many of these tests are required by regulations and, without exception, they are very animal intensive. In the EU – and it is taken over more and more by other regulatory authorities around the world – reproductive toxicity testing is required for all chemicals in 'wide-spread use' produced at more than 100 tonnes per year as this would imply a high exposure potential. As said before, many of such chemicals are UVCBs (e.g. fatty acid derivatives, fragrances, colourants, metal salts, petroleum hydrocarbons, enzymes). However, if all the UVCBs that are currently on the market would be tested with the available methodology for reproductive toxicity testing as required by law, it would not only be extremely animal-intensive, requiring large numbers of animals, but it would probably also yield unreliable results. As a consequence, there is a strong need for alternative testing methodologies and strategies.

A first research project I am working on at the division of Toxicology investigates the value of alternative testing strategies to investigate the developmental toxicity of a series of selected UVCBs and to validate the hypothesis, that the 3- to 7-ring polycyclic aromatic hydrocarbons (PAHs) that may be present in these complex, PAH-containing UVCBs are the sole inducers of prenatal developmental toxicity (PDT) which has been observed with these substances in *in vivo* studies. We know that some PAH induce prenatal developmental toxicity in animal studies. For instance, benzo[a]pyrene causes clear prenatal development toxicity in rats. On the other hand gas-to-liquid (GTL) substances, which are synthetic analogues of conventional petroleum substances but without any PAHs, cause no prenatal developmental effects whatsoever, while heavy fuel oil (HFO) components, which are petroleum substances with high concentrations of PAHs cause clear prenatal development toxicity in rats as well.

The first test system that we have applied to investigate this hypothesis is the mouse embryonic stem cell system. This system applies stem cells which are cultivated in hanging drops until they form embryoid bodies that are plated in 24-well plates, where they differentiate into beating cardiomyocytes. The development from undifferentiated cells into functional heart cells that actually contract in a synchronized way can be influenced by co-incubation of chemicals. The extent to which the chemicals under study inhibit the differentiation can be determined by simply assessing which percentage of embryoid bodies develops into beating cardiomyocytes. The differentiation from stem cells into contracting cardiomyocytes

is a highly complex process in which the cells not only differentiate morphologically but which also requires extensive communication between the cells. It is assumed that this complexity reflects to some extent what happens in a developing embryo [9, 10].

When we test a range of petroleum substances with varying amounts of PAHs and GTL substances without any PAHs in the embryonic stem cell system, we see, first of all, that there is no toxicity over a period of 5 days (the blue and red lines at the top of each graph, Figure 5a).

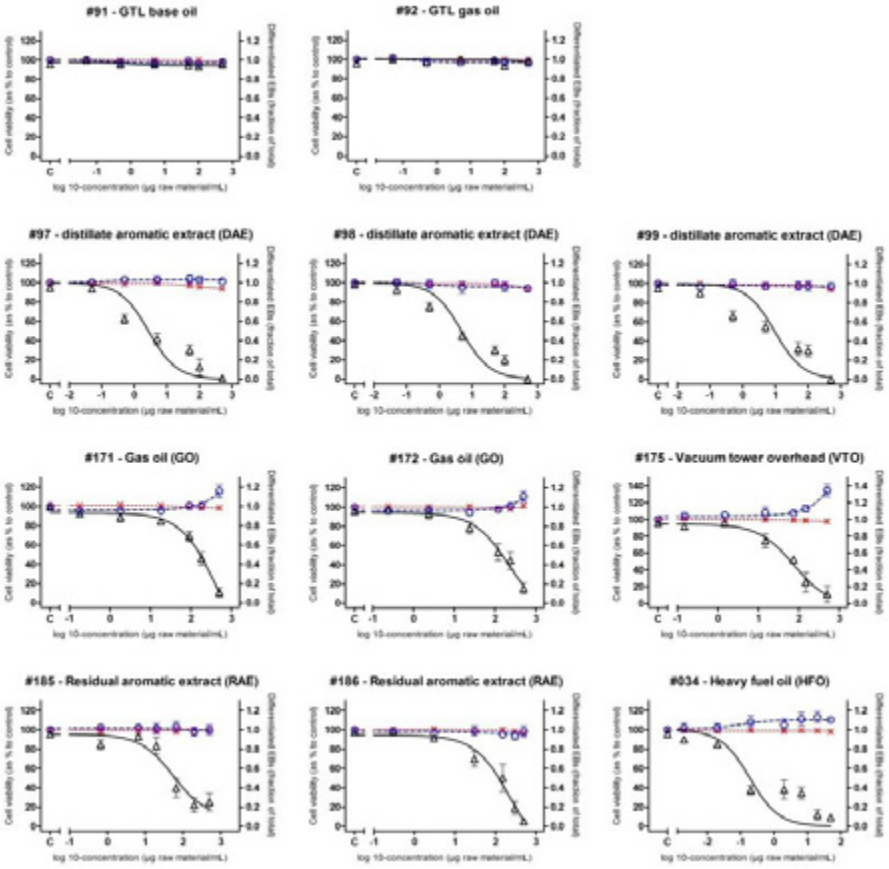

Figure 5 a. Concentration-dependent effects of DMSO-extracts of petroleum substances and GTL products on ES-D3 cell viability upon one-day (x and red line) and five-days (∘ and blue line) exposure and on inhibition of ES-D3 cell differentiation into contracting cardiomyocytes (Δ and black line). Results represent data from at least three independent experiments and are presented as mean ± standard error of the mean (SEM)[11].

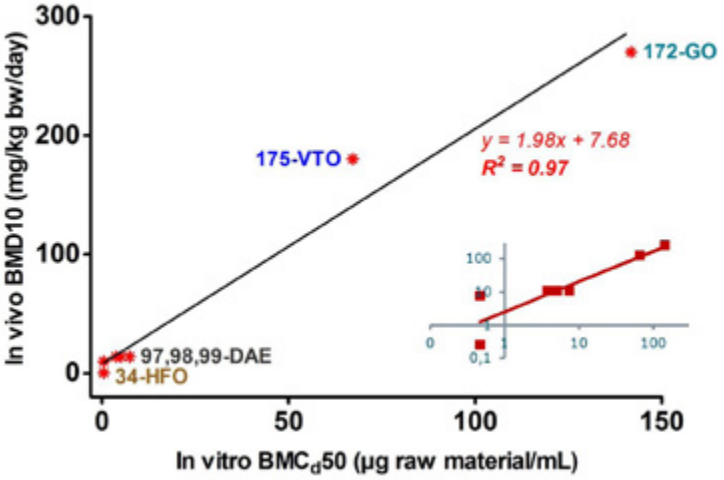

Figure 5 b. Correlation between in vitro BMCd50 values, obtained from the ES-D3 cell differentiation assay of the EST, and in vivo BMD[10] values, based on the increased incidence of fetal resorptions in pregnant rats. #34-heavy fuel oil (HFO), #97,98,99-distillate aromatic extract (DAE), #175-vacuum tower overhead (VTO), and #172-gas oil (GO)[11].

We also see that the GTL substances have no effect on differentiation, while all the petroleum substances have an effect on differentiation of the stem cells into beating cardiomyocytes. It is important to notice that the amount of material, the concentration, needed to yield 50% inhibition of differentiation varies over more than 3 orders of magnitude between the various petroleum substances. Moreover, the higher the concentration of PAHs in the substance tested, the lower the concentration needed to get 50% inhibition. When these 50% inhibition values are plotted against typical developmental reprotoxic parameters, such as the number of resorbed fetuses, as determined in vivo in rat studies, a very good correlation was found (Figure 5b). These results strongly suggest that the embryonic stem cell system is suitable to predict the prenatal developmental toxicity potency of petroleum substances and also supports the hypothesis that the PAHs present in these substances are responsible for these developmental effects [11].

Despite the fact that such an excellent correlation between the *in vitro* and *in vivo* potencies with regard to prenatal development toxicity was found with the embryonic stem cell test, we should be careful. The system appears to work really well with these petroleum and GTL UVCBs, but since prenatal developmental

toxicity is a highly complex endpoint, a *single system* like the embryonic stem cell test is unlikely to give reliable predictions for just any UVCB substance [12, 13]. In addition, there might be other developmental effects the system does not detect. This might for instance be due to the fact that the embryonic stem cell test system lacks significant metabolism. This can be overcome by including a metabolic system in the assay. We have already successfully done that and it did not have significant effects on the results obtained with the petroleum UVCBs. Because a single *in vitro* test may not reflect all possible endpoints of the complex developmental processes, it is important to include other tests systems in a battery of tests to get a better and more complete overview of possible prenatal developmental toxicity. Therefore we are also working on other *in vitro* test systems, such as the zebrafish embryo test and will also, at a later stage, include -omics studies on several human cell lines specific for human reproduction to better characterise the underlying mode of action [14, 15]. As also encountered for tests using the embryonic stem cell test, we will have to develop a way to introduce the substances into the system, which is not easy since they do not dissolve in aqueous systems. For the embryonic stem cell system we developed methodology to do this and we will investigate whether a similar approach also works with other test systems.

It is important to understand why we think that with a battery of *in vitro* test systems the complete picture of prenatal developmental toxicity of a class of substances can be tested. The reason is that we expect that the crucial pathways for reproduction and development are so fundamental that they are well preserved across species. This concept is illustrated by PAX mutations in a series of different species (Figure 6): when the PAX gene is knocked out in humans, the eye does not develop properly and the individual with the non-functional PAX gene is blind. In the mouse, knocking out the PAX gene has the same effect. Although fish eyes are quite different from mammalian eye, also in the zebrafish knocking out the PAX gene leads to the development of a non-functional eye. Surprisingly, in the nematode C. elegans, a tiny little worm without eyes, knocking out the PAX gene leads to deformation of the head at the place where you might expect an eye. Apparently, the PAX gene is responsible for essential developments in the head of all these species and this developmental function is very well preserved across all these species. Therefore, we expect to be able to find specific adverse-outcome pathways indicative for prenatal developmental by studying several systems and mapping the affected genes.

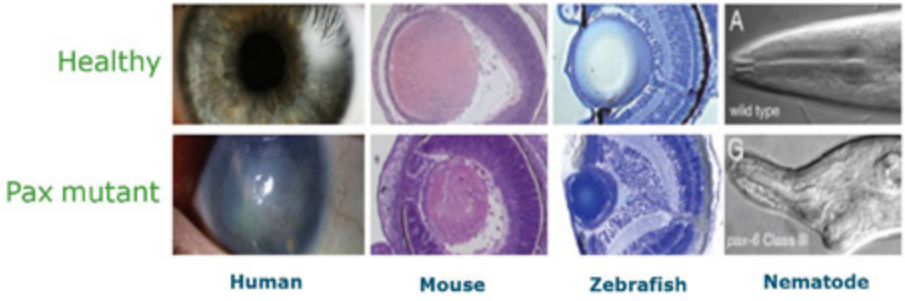

Figure 6. Evidence for conserved biology across species. The top panel shows the wild-type ("healthy") phenotype and the bottom panel shows the PAX6 ortholog mutation ("diseased") phenotype. In the human the PAX mutation causes aniridia (absence of iris), corneal opacity, cataract, glaucoma, and long-term retinal degeneration. For mouse, the mutants exhibit extreme microphthalmia with opacity of lens and cornea as well as iris abnormality. For zebrafish, the mutants express variable effects that consists of decreased eye size, reduced lens size, and malformation of the retina. In the nematode (C. elegans), which doesn't have an eye, the head is malformed (the spot where the eye would develop) (adapted from Washington et al.[16])

There is, however, one very important issue that needs to be taken into account in the development of these 'alternative tests' to replace the conventional testing in laboratory animals. Up till now, most 'alternative tests' to replace animal testing, have been designed for the development of medicines. The pharmaceutical approach, however, is fundamentally different from the toxicological approach needed for the safety testing of existing substances. The pharmaceutical alternative testing systems are designed to pick up potentially toxic substances at the expense of a relatively high rate of false positives. For the toxicological testing, as required by law for existing substances, a fundamentally different approach is needed that predicts negative outcomes with high certainty. We expect that this can be, at least partially, covered with a test battery approach, that covers a wide array of adverse-outcome-pathways. But this will require a fundamentally different approach with regard to specificity and sensitivity.

In addition to the development of an alternative approach for hazard testing, it is equally important to develop a different approach for the exposure assessment that allows the translation of the concentrations applied in alternative test systems to in vivo exposure data. To this goal, human biomonitoring data in combination with pharmacologically-based kinetic (PBK) models will be applied.

Human biomonitoring is a general term comprising the following subcategories of monitoring methods [17]:

- ***Biological monitoring***, which identifies the assessment of biomarkers of exposure. This type of biomarker is also referred to as 'internal dose' or 'body burden'. Typical examples of biological monitoring are the determination of metals (e.g. mercury, arsenic or lead) in blood or urine[18], the determination of unchanged substances (e.g. dioxins, PCBs or benzene) in adipose tissue, milk, urine, or blood, the determination of specific metabolites of a chemical (e.g. S-phenylmercapturic acid or trans,trans-muconic acid as metabolite of benzene) in urine[19, 20], or volatile compounds (unchanged substances or metabolites) and even metals in exhaled breath[21, 22].
- ***Biochemical effect monitoring***, which identifies the assessment of biomarkers of effective dose, which is also referred to as 'tissue dose'. Typical examples of biochemical effect monitoring include the determination of adducts of a specific chemical (e.g. ethylene oxide or polycyclic aromatic hydrocarbons) to DNA or a protein such as albumin or haemoglobin[23, 24]. It should be realised that both biological and biochemical effect monitoring solely provides evidence for exposure and cannot be interpreted in terms of health risk assessment without additional data on dose-response relationships.
- ***Biological effect monitoring***, which identifies the assessment of biomarkers of effect and gradually flows over into the assessment of clinical parameters or biomarkers of disease. Typical examples of biological effect monitoring include measurements such as cholinesterase activity in blood to monitor exposure to organophosphate or carbamate pesticides, zinc protoporphyrin or δ-aminolaevulinic acid to monitor exposure to lead, sister chromatid exchanges and other chromosomal aberrations to monitor exposure to genotoxic or clastogenic substances, and several specific forms of (micro)proteinuria to detect exposure to a variety of substances.

Various parameters assessed in biological effect monitoring may also be used in clinical practice and form a seamless continuum with *clinical monitoring* as a more or less arbitrarily set value may demarcate the shift from a minor biological effect to an effect that is considered clinically relevant. For example albuminuria may be used in biological effect monitoring (micro-albuminuria) as a biomarker of early renal function effects but it is also used as a clinical parameter, for instance, to assess renal function impairment in diabetics[25, 26].

In addition to biomarkers of exposure, of effective dose, of effect, and of disease, sometimes genotyping and phenotyping are referred to as *biomarkers of susceptibility*. Biomarkers of susceptibility are indicators of an inherent or acquired ability of an

organism to respond to the challenge of exposure to a specific substance.
Some examples are the expression of certain isoforms of cytochrome P450, glutathione transferases, or N-acetyltransferases. In addition, factors such as nutritional status, iron-status etc. may also be regarded as biomarkers of susceptibility.
These biomarkers differ from the other biomarkers in that they reflect potential interindividual differences in uptake and metabolism of chemicals and, consequently, potential differences in health risks[27-29]. Biomarkers of susceptibility may, like lifestyle factors, such as smoking and drinking behaviour, explain differences in biomarker results between individuals with identical exposure profiles (Figure 7).

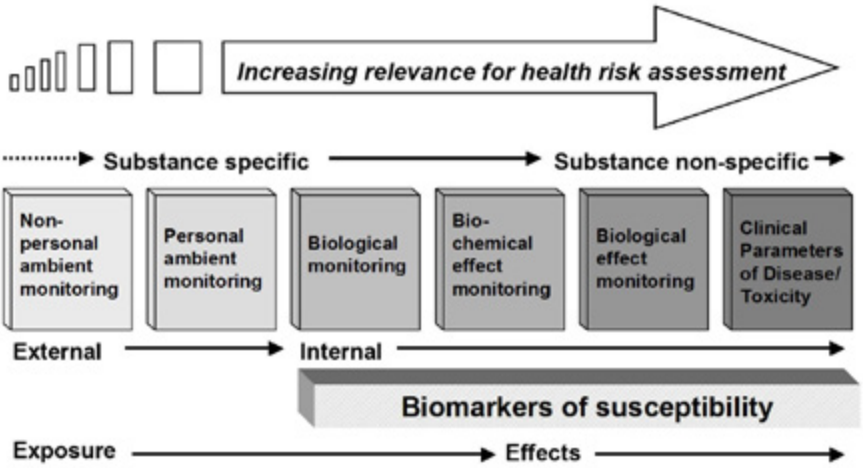

Figure 7. Monitoring techniques as part of the exposure–effect continuum in relation to human risk assessment[17].

If one takes a look at the potential ways to assess exposure to, for instance, food-contaminants, one can do a 'food basket analysis' which essentially means that the amount of the contaminant is determined in a representative diet. Slightly more accurate is a food questionnaire that gathers information from individuals about their diet and this information is combined with the concentration of the contaminant present in the food stuffs listed. Human biomonitoring measures the contaminant in the blood or urine of individuals and, of course, provides far more precise information. If the contaminant is a reactive substance that can bind to proteins or DNA, the determination of these adducts (*i.e.* biochemical effect monitoring) gives even more specific information about the amount of the contaminant that has reached the target tissues.

Biological effect monitoring measures early and, in most cases, reversible biological effects, which do not necessarily lead to health effects whereas abnormal clinical parameters are an expression of (beginning) disease. In general, in biological effect monitoring natural phenomena are measured for which a 'normal' background value may be established which may be influenced by various physiological and environmental factors that are not related to chemical exposure. This renders most methods of biological effect monitoring intrinsically non-specific.

The accuracy of the exposure determination as well as the relevance for health increases from non-personal ambient monitoring (i.e. static air monitoring, monitoring of drinking water, 'food basket' monitoring), via personal ambient monitoring (e.g. personal air monitoring, dermal exposure monitoring), to biological monitoring and biochemical effect monitoring. The relevance for health increases further with biological effect monitoring and clinical effect monitoring, but with a loss of specificity with regard to the chemical (or physical) factor associated with the health effect. In general, for health risk assessments biological monitoring and biochemical effect monitoring provide the best and most reliable information both in terms of exposure and potential health effects related to certain exposures. For that reason, in my research I concentrate on biological monitoring and biochemical effect monitoring. Both methods should be regarded as exposure assessment and are specific for the substance that is being measured, just like most ambient monitoring methods. In fact, in biological monitoring as well as in biochemical effect monitoring each individual serves as its own dose monitor.

The approach that will be followed can be illustrated by an example from the food chain. The modern food chain may contain several generally toxic, genotoxic or reprotoxic contaminants as a result from environmental pollution and/or processing. Examples are again the PAHs but also acrolein, acrylamide, glycidyl esters, furans, and mineral oil residues. In safety assessments for food contaminants, the largest uncertainty resides often in the actual exposure levels. As indicated, the more traditional ways to assess exposure by food basket analysis and/or food frequency questionnaires is very unreliable and human biomonitoring gives much better estimates. In addition, where the traditional methods fail to link to the *in vitro* data generated in the alternative test methods, biomonitoring data can potentially be linked to these data by application of reversed dosimetry PBK models. PBK models have been mentioned now several times. Essentially, these are mathematical models that describe all the essential parts of the body, such as the liver, lungs, kidneys etc. and the blood flows between mathematical equations in combination with the way substances are being processed. They have been shown to be very powerful tools in predicting the metabolism of these substances and also the concentrations of these

substances and their metabolites in the blood, urine, saliva etc. following oral, dermal or inhalation exposure to these substances. This process is called 'dosimetry'. In 'reverse dosimetry', the same PBK models are used to use the concentrations of substances and their metabolites in the blood, urine, saliva – that is typical biomarkers of exposure – to predict the actual exposure that has occurred[30-32].

For any approach of human biomonitoring, the choice of an appropriate biomarker is essential. For the PAHs there are several candidates available of which some, such as 1-hydroxypyrene and the hydroxyphenanthrenes, are routinely applied[33, 34]. For these biomarkers PBK models are already available and in addition relatively large databases with biomarker data[35]. For other potential food contaminants suitable biomarkers are not readily available. Many food contaminants of concern are electrophilic in nature or have electrophilic metabolites. As a consequence they may react with glutathione and be excreted as stable mercapturic acids which have been shown to be in general very suitable biomarkers of exposure[36, 37]. For compounds such as acrylamide and acrolein it has already been shown that stable mercapturates are being formed, but suitable PBK models need to be developed and databases constructed. Any PBK model must be validated against available *in vivo* data and can then be applied to convert available urinary biomarker data to exposure levels in the human population. Modern statistical techniques, such as Monte Carlo simulations, may be used to extend the available data to a larger population[38]. The exposure assessment by human biomonitoring can be used to compare to exposure scenarios, such as those developed by authoritative bodies (EFSA, JECFA) but also to bring existing risk assessments up to date provided that reliable hazard data are available.

If the hazard data are obtained from alternative, *in vitro* systems, the concentrations in these systems need to be correlated to the actual exposures as measured by human biomonitoring to assess the human health. Although there are several models and data available, thus far the integration between the various approaches is lacking. Essentially, in traditional human health risk assessments results from *in vivo* experiments in laboratory animals are translated to the human situation by application of assessment factors, sometimes in combination with modelling. The same approach cannot be applied to *in vitro* results. However, it seems a valid assumption that the effective *in vitro* concentration that triggers a specific toxic effect in an alternative test system correlates with the internal concentration in the human body that elicits a similar effect at the receptor site. In addition, it is commonly accepted that the concentration at the receptor site is correlated with the concentration in the blood. This concept basically allows to link concentrations applied in alternative *in vitro* test systems to the blood concentrations in exposed humans using kinetic models. Subsequently, the exposures to the chemical can be

calculated using reverse-dosimetry PBK models as a basis for human health risk assessment.

This approach will be used for the embryonic stem cell data. We will design kinetic models which link the *in vitro* concentrations of relevant PAH in the embryonic stem cell test to human biomarker concentrations and subsequently to the corresponding external dose levels thus allowing animal-free health risk assessment of reprotoxic petroleum substances. In previous projects we have already built PBK models for PAHs (e.g. pyrene, benzo[a]pyrene) and we can extend these models, using primarily physico-chemical data to any other PAH of interest including PAHs that may pose a risk and those that are very unlikely to do so. Once this is done, we can progress to more refined models with newly generated data to get a more precise human health risk assessment (Figure 8).

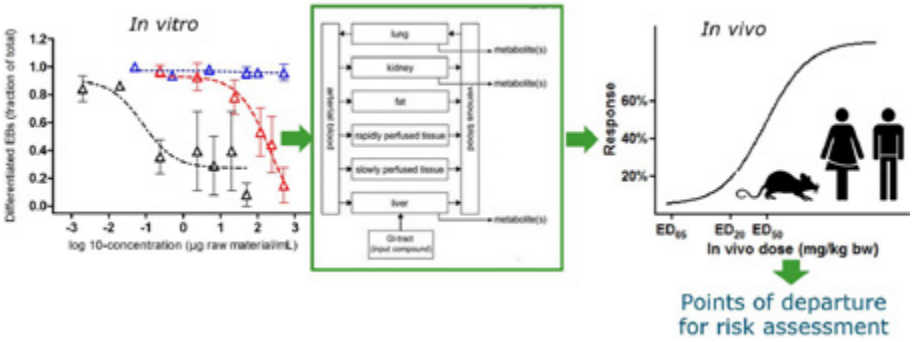

Figure 8. The role of physiologically-based kinetic (PBK) modelling in translating in vitro concentrations to in vivo concentrations which can subsequently be used as point of departure in the human health risk assessment process.

Overall, the research we are undertaking at the moment aims at developing better alternative methods for hazard assessment to reduce of the number of animals used for toxicity testing and obtain more relevant data by using human or humanised cell systems. At the same time we aim at collecting better and more relevant exposure data by application of human biomonitoring. In combination with PBK modelling this will lead to more relevant human health risk assessment of exposure to (complex) chemicals faster, cheaper, and using much fewer animals (Figure 9).

Figure 9. The proposed alternative human health risk assessment scheme based on animal-free hazard assessment and internal exposure assessment in combination with physiologically-based kinetic (PBK) modelling.

At the very end of this talk, I would like to take the opportunity to say a few more personal things in Dutch.

Het is natuurlijk zo dat ik hier vandaag alleen maar kan staan en deze voordracht geven omdat veel mensen daar in de loop van de jaren aan hebben bijgedragen. Een aantal van hen wil ik hier in het bijzonder noemen. Allereerst is dat mijn leermeester en promotor Prof. Gerard Mulder. Gerard, wellicht heb ik niet zo heel veel toxicologie van jou geleerd in de tijd dat ik op het Sylvius Laboratorium in Leiden rondliep, maar één ding heb ik zeer zeker van jou geleerd in die tijd en dat is het schrijven van een fatsoenlijk artikel. Later, na mijn promotie bij jou, heb ik nog iets heel belangrijks van jou geleerd, namelijk het goed en efficiënt voorzitten van een vergadering. Daarbij denk ik dan aan de lange periode – inmiddels al ruim 17 jaar – dat wij beiden in de Gezondheidsraad zaten waar jij menige commissievergadering hebt voorgezeten.

Vervolgens wil ik twee personen in één adem noemen, namelijk Dr. Wim Tordoir en Dr. Nico van Sittert. Wim, jij hebt me meer dan 27 jaar geleden aangenomen bij Shell, en Nico, jij was m'n eerste baas bij Shell en later nogmaals. In de loop van de tijd heb ik op verschillende tijden aan jullie gerapporteerd. Hoewel jullie een uiterst verschillende stijl van leiding geven hadden, hadden jullie één ding gemeen: jullie beiden hadden een groot vertrouwen in m'n capaciteiten en lieten me vrijwel ongestoord m'n gang gaan. Alles wat ik toen geleerd heb, en de talrijke projecten die

ik op die manier heb kunnen uitvoeren, hebben in grote mate bijgedragen aan het feit dat ik hier vandaag in deze hoedanigheid sta.

Ook Prof. Ivonne Rietjens hoort in dit rijtje thuis en dat is niet alleen omdat ik van haar vakgroep onderdeel uitmaak. Ivonne, ik denk dat het al meer dan vijf jaar geleden is dat je er op aandrong om te overwegen bij jou in de vakgroep te komen. Het proces werd een paar jaar geleden in gang gezet, maar dat ging bepaald niet altijd van een leien dakje. Ondanks de strubbelingen met zowel Shell als de WUR, bleef jij er in geloven en onverminderd enthousiast en uiteindelijk is het nu zo ver gekomen. Ik denk dat we samen hele mooie dingen gaan doen.

Ik ben heel blij dat, ondanks haar hoge leeftijd, mijn moeder hier bij kan zijn. Mama, u en papa hebben altijd het belang van goed onderwijs onderkend en kosten nog moeite gespaard om alle zeven kinderen een goede opleiding te geven. Op een gegeven moment waren er zelfs vijf van ons tegelijk aan het studeren, maar ik kan me niet herinneren dat daar ooit een probleem van gemaakt is – in elk geval niet naar ons kinderen toe. Verder heeft u altijd een groot vertrouwen en trots in mijn kunnen ten toon gespreid en daarom doet het me heel erg veel deugd dat u hier vandaag bij kunt zijn.

Tenslotte, wil ik Aly hier noemen, al meer dan 28 jaar mijn echtgenote en levensgezellin. Ik heb een hoop domme dingen gedaan in m'n leven, en gelukkig ook een aantal verstandige dingen. Eén van de allerverstandigste dingen die ik ooit gedaan heb, is jou ten huwelijk vragen. Ik zou dat vandaag onmiddellijk weer doen, maar gelukkig hoeft dat niet, want het is maar de vraag of je weer ja zou zeggen... Toen Wageningen in beeld kwam zei je wat zuinigjes: "Mmm, dus je gaat voortaan vijf dagen per week voor Shell werken en twee dagen voor de universiteit ?". Inderdaad heeft dat scenario wel enige mate van waarschijnlijkheid. Of dat de bestaande situatie heel fundamenteel gaat veranderen weet ik niet, maar het moge duidelijk zijn dat ik nooit had kunnen doen wat ik gedaan heb – of dat nou m'n werk bij Shell of hier in Wageningen is, of het beklimmen van de Seven Summits – zonder jou aan mijn zijde. Ik hoop van harte dat dit nog vele jaren het geval zal zijn.

Ik heb gezegd.

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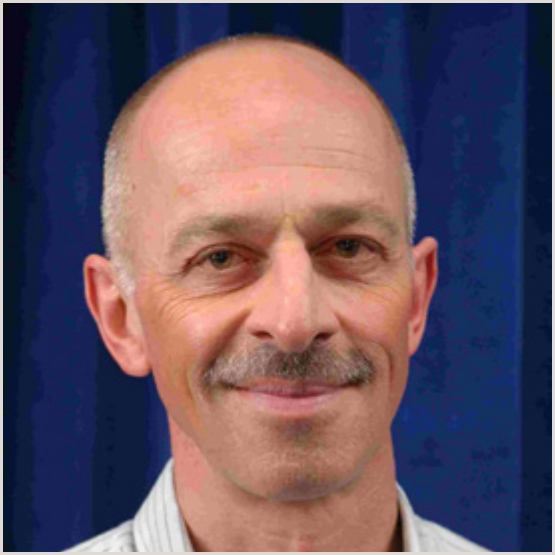

Prof.dr Peter J. Boogaard

'Classical health risk assessment relies on combining test data from experimental animals with external exposure measurements via the air, water, or food. This has severe limitations as it requires many animals and may poorly predict the human situation. A new paradigm is proposed that relies on human in vitro test systems and biomonitoring (internal exposure assessment e.g. in urine or blood) in combination with mathematical models. This leads to more reliable, animal-free health risk assessments with greater relevance for humans.'

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